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## Mirror-changing confocal optical device

### Technical field

- 5 This concerns a confocal optical device comprising a means for changing the beamsplitter that splits the illuminating beam directed towards the observed object from the beam to be detected coming from the observed object.

### Prior art

- 10 Figure 1 depicts part of a confocal optical device according to the prior art. A light beam originating from a laser 308 is broadened and collimated by lenses 300, 301. The illuminating beam FE having passed through the lens 301, which is substantially parallel, is then reflected by the partially reflective mirror 302 and is then sent back towards the lens 304 which can be the objective of the microscope or an intermediate lens. The lens 304
- 15 focuses the illuminating beam originating from a point of the array 300 on an illuminated point 309 on the image plane 307 which can be an intermediate image plane or directly an observed object. The beam to be detected FD returning from the illuminated point passes through the lens 304 again in the reverse direction and passes through the partially transparent mirror 302. It passes through the lens 305 and reaches a microscopic hole 306
- 20 which has a filtering role and is placed in a focal plane of the lens 305. In the figure the partially transparent mirror 302 is in an afocal zone, that is to say the illuminating beam and the beam to be detected are substantially parallel in this zone. However, in confocal microscopy, the partially transparent mirror is not necessarily placed in an afocal zone. For example it can also be placed in immediate proximity to the microscopic hole. The partially
- 25 transparent mirror 302 can typically be a dichroic mirror splitting the excitation and emission wavelengths, in the case of a microscope operating in fluorescence mode, or a beamsplitter that is neutral as regards wavelength, for observing the light reflected by an object to be observed.

- For the system to work, the illuminated point 309 must be conjugate with the
- 30 microscopic hole 306. But the accuracy of positioning of the mirror 302 is insufficient to allow such a conjugation to be reproduced reliably when the mirror is replaced by another one or removed and then put back in place. This is because any inaccuracy in positioning the mirror modifies the direction of the illuminating beam reflected by the mirror, and

consequently moves the illuminating point which ceases to be conjugate with the microscopic hole. Exchange of the mirror 302 is necessary for example, if it is a dichroic mirror, for changing excitation wavelength. There can also be several distinct illuminating light paths which arrivreach the afocal zone and are each superimposed on the beam to be  
5 detected by a partially transparent mirror. In this case, each mirror must be removable, so that an illuminating light path can be used without being interfered with by the mirror corresponding to another illuminating light path.

For simplification, Figure 1 does not depict the scanning device which can for example be a pair of galvanometric mirrors or a device for translational movement of the  
10 sample. Figure 1 can be adapted to the case of multipoint illumination by replacing the lens 300 by an array of microlenses, and replacing the microscopic hole 306 by an array of microscopic holes.

The problem of the loss of conjugation between the focusing point of the illuminating beam in the object, which is conjugate with the virtual focusing point of the  
15 illuminating laser, and the microscopic filtering hole, during a change of dichroic mirror, is usually solved in various ways:

- a) by considerably magnifying the image forming in the plane 306 so as to replace the microscopic hole by a hole with larger dimensions and by placing the dichroic mirror in proximity to this hole and not in an afocal zone. This solution appreciably lengthens the  
20 optical paths and is not transposable to the case of an array of microscopic holes (multipoint illumination). This is because, in the latter case, magnifying the image necessitates magnifying the entire array of microscopic holes, which leads to dimensions of the array which are incompatible with the normal dimensions of a confocal device.
- b) by providing a system for readjusting the position of the microscopic hole.
- 25 c) by combining the preceding solutions in order to avoid readjustments that are too large or too frequent, without excessively lengthening the optical path.
- d) by making the illuminating beam pass through the microscopic hole, and placing the dichroic mirror before the microscopic hole on the path of the illuminating beam, and therefore after the microscopic hole on the path of the beam returning from the object. This  
30 solution simplifies the system but does not make it possible to adjust the size of the microscopic hole without also affecting the illuminating beam, nor does it make it possible to correct chromatic aberration differences between the illuminating beam and the beam to

be detected returning from the observed object. It therefore results in a reduction in the quality of the images obtained.

#### Description of the invention

5           The aim of the invention is to solve the problem of loss of conjugation between the focusing point 309 of the illuminating beam and the microscopic hole 306 at the time the beamsplitter 302 is exchanged, while avoiding the shortcomings characterising the techniques mentioned above, and in a way that is compatible with the use of multipoint illumination.

10           The invention consists of a confocal optical device for illuminating at least one illuminated point using an illuminating beam coming from an illumination source and focused on the illuminated point, and for focusing on a microscopic hole a beam to be detected coming from the illuminated point, comprising a beamsplitter passed through by a first beam and reflecting a second beam, one of the first and second beams being the  
15 illuminating beam, and the other being the beam to be detected, the beamsplitter being exchangeable, the device being characterised by the following facts:

- it comprises a redirection mirror substantially parallel to the beamsplitter and reflecting the second beam,
- the beamsplitter and the redirection mirror are attached to one another, so that the  
20 redirection mirror and the beamsplitter together constitute a splitter unit, which is exchanged all in one piece at the time the beamsplitter is exchanged.

For example, the beamsplitter can be a dichroic mirror and the redirection mirror can be a reflective-only mirror. The beamsplitter can also be a partially transparent mirror (neutral beamsplitter) or a reflective-only mirror.

25           If the beamsplitter were positioned independently of the redirection mirror, an error in positioning the beamsplitter would affect the direction of the second light beam, which could therefore not be considered as reproducible when the beamsplitter is moved outside the optical path and then put back in place. The fact that the two mirrors are attached has the consequence that the direction of the second beam at the output of the splitter unit is  
30 not affected by errors in positioning the splitter unit assembly. This is because, after reflection on two mirrors parallel to one another, a light beam finds its initial direction again exactly, irrespective of the angle between the beam and the mirrors. It is also confirmed that an error in positioning the splitter unit translationally does not affect the

light beams, whereof neither the position nor the direction are affected at both the input and output of the splitter unit.

However, the presence of a redirection mirror attached to the beamsplitter and parallel to the beamsplitter is not sufficient to eliminate all sensitivity of the system to errors in positioning the splitter unit thus formed. This is because an error in positioning the splitter unit rotationally results in a corresponding translation of the second light beam, although the direction of this beam remains constant. This translation can result in a loss of optical conjugation between the illuminated point and the microscopic hole.

According to the invention, this problem is solved by placing said beamsplitter and redirection mirror (therefore the splitter unit) in an afocal zone, in which the illuminating beam and the beam to be detected are substantially parallel.

Because the splitter unit is placed in an afocal zone, a direction of the beam at the splitter unit corresponds to a point in a plane where the beams are focused, and therefore the position of such a point (and consequently the conjugation between the focusing point of the illuminating beam and the microscopic hole) is not affected by errors in positioning the splitter unit, both rotationally and translationally.

The invention is adapted to both single-point systems and multipoint systems. However, in the case of multipoint systems, the existing techniques are more difficult to apply, perhaps even impossible in the case of technique (a) mentioned above. The invention is therefore particularly useful for multipoint systems, in which case the optical device according to the invention comprises means for illuminating a plurality of illuminated points using a plurality of illuminating beams, and for focusing on a plurality of microscopic holes a plurality of beams to be detected each coming from an illuminated point, said beamsplitter being passed through by a plurality of first beams, said beamsplitter and redirection mirror reflecting a plurality of second beams, said first beams being the illuminating beams and said second beams being the beams to be detected, or said first beams being the beams to be detected and said second beams being the illuminating beams.

The beamsplitter can be for example a dichroic mirror or a partially transparent mirror neutral as regards wavelength. The redirection mirror is preferably a totally reflective mirror.

In order for it to be possible to exchange the splitter unit, the device according to the invention preferably comprises a plurality of splitter units each consisting of a

beamsplitter and a corresponding redirection mirror, and a means for alternately placing one or another of the splitter units on the optical path. This means can be for example a slider or a wheel turning about its axis.

If the beamsplitter and redirection mirror are not perfectly parallel, the direction of the second beam at the output of the splitter unit can be slightly modified with respect to its direction at the input of the splitter unit. So that several splitter units can be exchanged without loss of conjugation between the illuminated point and the microscopic hole, it is necessary that all the exchangeable units generate the same beam direction variations, with very great accuracy. This is difficult to achieve with splitter units comprising several assembled components. According to a preferred version of the invention, the beamsplitter and the redirection mirror are placed on two opposite faces of a parallel window. This window is disposed so that:

- the optical path of the second beam comprises successively a first passing-through of the parallel window, a reflection on a first mirror, a second passing-through of the parallel window, a reflection on a second mirror, and a third passing-through of the parallel window, one of the first and second mirrors being the redirection mirror and the other being the beamsplitter,
- and so that the optical path of the first beam comprises a passing-through of the parallel window and a passing-through of the beamsplitter.

The beamsplitter and the redirection mirror are for example made by depositions of thin coatings on the parallel window. The window must be sufficiently thick to allow effective splitting of the light beams. Under these conditions, good parallelism of the faces of the windows constituting several splitter units is sufficient to ensure the interchangeability of these units. This is easily achievable in an optical workshop. If the windows constituting mutually exchangeable splitter units do not have perfectly parallel faces, the angle between these faces must be the same for all the mutually exchangeable splitter units.

The parallel window allowing splitting of the light beams forms an integral part of the invention, in the same way as the confocal device as a whole. The invention therefore also consists of a splitter unit intended for a confocal optical device, characterised by the fact that it consists of a parallel window,

- a first face of said window comprising a first area on which a dichroic or partially reflective mirror is made by deposition of at least one thin coating, intended to be passed through by a first light beam and to reflect a second light beam,
- the first face of said window comprising a second non-reflective area, intended to be  
5 passed through by the second light beam,
- a second face of said window, opposite to the first face, comprising a third area on which a redirection mirror is made by deposition of at least one thin coating, intended to reflect the second light beam,
- the second face of said window also comprising a fourth non-reflective area, intended to  
10 be passed through by the first light beam and by the second light beam.

In fact such a splitter unit makes it possible to split a first light beam from a second light beam without altering the direction of these beams. It is intended as a priority to be used in a confocal microscope, but can also be used in other devices requiring the reproducible exchange of a splitter unit not altering the direction of the light beams.

#### Brief description of the figures

Figure 1 shows a confocal optical device according to the prior art. Figure 2 shows a confocal optical device according to the invention. Figure 3 shows in perspective a splitter unit according to the invention. Figure 4 shows the same splitter unit in cross-  
20 section. Figure 5 shows several splitter units associated within a mirror-changing slider. Figure 6 shows in cross-section another type of splitter unit. Figure 7 shows a slider associating several splitter units of the type depicted in Figure 6. Figure 8 shows a preferred type of splitter unit. Figure 9 shows the association in a slider of several splitter units of the type depicted in Figure 8. Figure 10 shows a single-point confocal device  
25 according to the invention using the splitter unit of Figure 8. Figure 11 shows a multipoint confocal device according to the invention using the splitter unit of Figure 8.

#### Embodiments

Figure 2 depicts a simple embodiment of the device according to the invention. The  
30 system is identical to that of Figure 1, but the beamsplitter 302 has been replaced by the splitter unit consisting of the beamsplitter 321 and the redirection mirror 320 and situated in an afocal zone.

Figures 3 and 4 show one particular embodiment of the splitter unit. This comprises a glass component 403 comprising a surface 401 on which the beamsplitter is made, and a surface 400 on which the redirection mirror is made. It is necessary to use a second glass component 402 so as not to disrupt the course of the part of the beam that passes through the beamsplitter. The optical paths of the illuminating beam FE and detection beam FD have been depicted by dotted lines. As shown in Figure 5, several independent splitter units 410, 411, 412 can be associated in a slider 414 allowing them to be brought successively into the optical path.

The slider can possibly comprise only a single splitter unit 412 besides the assembly 413, and in this case it is used simply to position or remove the splitter unit. As a general rule, it is desirable to have very great accuracy in the parallelism between the faces 400 and 401 and in the parallelism between the faces 404 and 406 so as to avoid two distinct splitter units directing the beam in different directions. However, in the case where only one splitter unit 413 is used, this accuracy is less essential since it has little effect on the reproducibility of the conjugation properties when the same assembly is put in place, taken out and put back in place. The faces 404, 406, 405 must also be perfectly parallel to one another.

Figure 6 shows another type of splitter unit according to the invention. This comprises a support 500 with holes in it for letting the light beam pass through, on which a beamsplitter 501 and a redirection mirror 502 are positioned. The illuminating beam passes through the hole 503, is reflected by the redirection mirror 502, is reflected by the beamsplitter 501, and leaves the device via the hole 504. The mirrors 501 and 502 are made by deposition of a reflective coating on glass windows. They are held resting on the surface of the support 500 by spring steel elements, for example 505 and 506, which apply pressure on the periphery of the mirrors. They can also be fixed by a thin layer of adhesive. If the support 500 is itself made of glass, "molecular bonding" is also possible. Several splitter units can be associated in a single slider. In this case, for these assemblies to be easily interchangeable, it is necessary for the parallelism between the surfaces on which the redirecting mirror 502 and the beamsplitter 501 are respectively positioned to be made with extreme accuracy. This constraint can be lessened by making in a single component several supports of the type shown by Figure 6. For example Figure 7 shows a multiple support 520, comprising first partially transparent mirrors 511 to 514 corresponding to the mirror 501 of Figure 6, a hole 510, and holes 521 to 525 corresponding to the hole 503 of Figure



6. Good flatness of the surfaces of the multiple support thus made is in fact sufficient to obtain good reproducibility of the direction of the beam, even when several partially transparent mirrors are used successively and when a slight parallelism defect remains between the surfaces of the two mirrors 501, 502. However, putting the beamsplitter and  
5 redirection mirror in position on their resting surfaces remains difficult to carry out with the necessary accuracy.

The sliders can be motorised. However, it is also possible to mount several splitter units on a wheel turning about a spindle, which makes it possible to reduce the friction compared with a slider system and therefore facilitate motorisation.

10 Figure 8 shows a preferred embodiment of the splitter unit making it possible to manufacture without excessive technical difficulties an independent and easily interchangeable splitter unit. In fact, the embodiments described previously are difficult to implement with the accuracy necessary for distinct splitter units to be interchangeable without disrupting the point-to-point conjugation relationships between the different image  
15 planes of the device of the invention. The splitter unit depicted in Figure 8 solves this problem. It consists of a sufficiently thick parallel window 600, on which the beamsplitter 602 is made by deposition of a thin coating (for example a multi-coating deposit in the case of a dichroic mirror) and the redirection mirror 603 is also made by deposition of a thin coating (typically a metallic coating or a multi-coating deposit). The illuminating beam FE  
20 enters the parallel window via an area 604 which can be antireflection coated, passes through it and reaches the redirection mirror 603 which reflects it. It again passes through the parallel window and is reflected by the beamsplitter 602. For a last time it passes through the parallel window which it leaves via the area 601 which can be antireflection coated. The beam to be detected FD enters the window via the area 601, passes through it,  
25 reaches the mirror 602 and passes through it. As shown in Figure 9, several splitter units 701, 702, 703, 704 of the type depicted in Figure 8 can be associated in a slider 700 making it possible to change from one unit to another. The parallel window can typically be made of glass and the making of two perfectly parallel faces on a glass window does not pose any technological difficulties. The dimensions of the parallel window depend on the width of  
30 the beams and can typically be 15 mm (thickness separating the faces bearing the beamsplitter 602 and the mirror 603) x 15 mm (width) x 45 mm (length). This solution therefore makes it possible to obtain at an acceptable cost easily interchangeable splitter units intended for example to be mounted on wheels or sliders.

This device generates a lateral shift of the light beams which can be compensated for by a corresponding shift of the lenses of Figure 2. Figure 10 depicts, by way of example, Figure 2 modified for the case of use of the parallel window 600 described in Figure 8. The same numbering as in Figure 2 has been kept, adding the numbers 602 and 603 used in Figure 8 and corresponding respectively to the mirrors 321 and 320 of Figure 2. Figure 11 depicts a preferred embodiment of the invention in the case of multipoint illumination and use of the splitter unit described in Figure 8. A collimated laser beam 800 is split by the array of microlenses 801 into a plurality of illuminating beams FE. The figure depicts one of these beams in solid lines and another in dotted lines. The illuminating beams then pass through the lens 802 after which each illuminating beam is substantially parallel. They are reflected by the mirror 803. The illuminating beams then reach the splitter unit consisting of the parallel window 600. They enter the window, are reflected by the mirrors 603 and 602, and then leave the window. They pass through the objective 804 and are focused on illuminated points of the object 807. The beams to be detected FD coming from the illuminated points then pass through the objective 804, pass through the parallel window 600 and the mirror 602, pass through the lens 805 and are focused on the holes of the array of microscopic holes 800. The scanning device, which can typically be a galvanometric mirror placed between the parallel window and the objective 804, has not been depicted.

In the figures, it has always been the beamsplitter that is passed through by the beam to be detected. It is also possible that it is the beam to be detected that is reflected and the illuminating beam that passes through the beamsplitter, which does not alter the nature of the invention.

#### Industrial applications

The device described allows rapid and reliable exchange of the dichroic mirror in confocal microscopes, in particular multipoint ones. This exchange is for example necessary during the observation of cells marked with several fluorescent markers, in order to successively obtain images corresponding to each marker.